

INSULIN REGULATES PLASMA GHRELIN CONCENTRATIONS in STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Ghrelin, an orexigenic peptide produced in the stomach has shown to elicit peripheral actions including regulation of pancreatic β -cell function. The aim of the study is to clarify the regulation of plasma ghrelin concentrations by insulin in streptozotocin (STZ) induced diabetic rats. Adult male Wistar rats were divided into control and three experimental groups as each group had 7 rats (n=28). To investigate the role of ghrelin in the hyperphagic response to uncontrolled diabetes, experimental groups of rats were injected once daily for 7 days with either STZ (70 mg/kg i.p.) or insulin subcutaneously (5-7 U). Plasma insulin, ghrelin and glucose concentrations were measured. STZ-induced diabetic rats were markedly hyperphagic as accompanied by hyperglycemia. Treatment of diabetic rats with insulin reversed these changes. STZ- induced diabetic rats had higher plasma ghrelin concentrations than control rats. Ghrelin levels were attenuated by the subcutaneous injection of insulin (5-7 U over 7 days). Insulin treatment also partially reversed hyperphagia observed in STZ- induced diabetic rats and there was a decrease in plasma ghrelin concentrations compared with STZ-INS pair fed rat. The results indicate that insulin treatment reverses elevated plasma ghrelin concentrations in STZ- induced diabetic rats suggesting the pathophysiological significance of ghrelin in diabetes.

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Introduction

Ghrelin, a novel 28 amino acid peptide, is the endogenous ligand for the growth hormone secretagogue receptor.¹ The stomach is the primary source of ghrelin, but it is also detected in small amounts by the intestines, kidneys, pituitary, hypothalamus and placenta.^{2,3} In addition to stimulating growth hormone secretion^{4,5}, ghrelin has profound orexigenic properties.^{6,7} Therefore, a role for ghrelin in regulating food intake and body weight has been proposed.^{8,9}

Consistently, ghrelin elicits many peripheral actions, including regulation of pancreatic β -cell function and influence on glucose metabolism.^{10,11} In the endocrine pancreas, ghrelin-producing ϵ -cells, suggests a role in islet function.¹² However, the regulation of plasma ghrelin concentration is still largely unknown.

Insulin deficient diabetes induced in rodents by the beta-cell toxin streptozotocin (STZ) is characterized by marked hyperphagia.⁴ In STZ-induced diabetic rats, postprandial ghrelin concentrations were found to be higher than in control rats.¹³ Ex vivo studies showed that ghrelin secretion from the stomach was suppressed by insulin infusion^{14,15}, indicating that insulin regulates circulating ghrelin levels. On the other hand, ghrelin has been reported to impair insulin sensitivity and secretion.¹⁶

Therefore, based on these evidences, we hypothesized that insulin decreases plasma

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ghrelin levels. Thus, the effect of insulin on ghrelin secretion is uncertain. This study sought to clarify the regulation of plasma ghrelin concentrations by insulin in STZ induced diabetic rats.

Methods

Chemicals and Drugs

Insulin (Humulin-R) were purchased from Eli Lilly (Indianapolis, IN). Streptozotocin (STZ) were purchased from Sigma- Aldrich Corporation (St. Louis, MO).

Animals and Treatment

The committee for animal experiments of the Dicle University Medical Research Center (Diyarbakır, Turkey) gave its approval for the project. All experiments were carried out according to local guidelines for the care and use of laboratory animals and the guidelines of the European Community Council for experimental animal care. Every effort was made to minimize animal suffering and the number of animals used. Experiments were carried out on male Wistar adult rats (n=28) weighing 180-210g. All animals were allowed free access to standart rat food and tap water ad libitum before the experiments. All the rats housed under standart conditions in a room under a constant 12 h light /dark cycle with a humidity $50 \pm 10\%$. All the experimental procedures were carried out between 07.00 and 19.00 a.m.

Experimental Design

Rats were divided into four groups (Control, STZ, STZ-İNS, STZ pair-fed) and sorted by body weight and food intake during the 1 week acclimation period. Each group contained 7 rats, and STZ (70 mg/kg i.p.) was administered as one-shot bolus after dissolved in the phosphate buffered saline (PBS) for 7 days before the beginning of the feeding and insulin treatment except control group.

The food intake of the latter group matched that of the STZ group to exclude the secondary effect of the insulin- induced feeding suppression. After the food consumption by the STZ- İNS group was analyzed, the same amount of food was administered to the pair-fed group. For pair-feeding regimen, we calculated the daily

food intake for the insulin- treated group.

On the basis of a preliminary study of food consumption during day and night, one-fourth of the total amount was provided in the morning (07.00), while the remaining three-fourths was provided before dark (19.00). Rats were injected at 19.00 once daily for 7 days with either PBS or insulin subcutaneous injection (5 -7 U).

The cumulative food intake was measured once daily for 7 days, and each group was measured for changes in body weight and plasma ghrelin.

Measurement of Food Consumption and Body Weight

The cumulative 24-h daily food intake of each rat (all males, 7/group) and its body weight were measured. Each rat was housed alone during the monitoring period under the ambient conditions described in animals.

Plasma Glucose and Hormone Measurement

During the days of insulin treatment, blood samples were taken through the jugular veins at 18.00 which were then separated into plasma and immediately frozen at -20°C until assayed. The blood samples were placed in Vacutainer tubes containing EDTA, and immediately after blood collection, the tubes were rocked several times to prevent coagulation. Next, the blood was transferred from Vacutainer tubes into centrifuge tubes containing aprotinin and gently rocked several times to inhibit proteinase activity. The samples for detecting the plasma glucose and insulin levels were assayed using commercial kits (Eiken Chemical, Tokyo, Japan); the samples for plasma ghrelin were also measured with commercial kits (Phoenix Pharmaceuticals, St. Joseph, MO).

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows statistical package, version 10.0 (SPSS Inc. Chicago, IL, USA). All results are expressed as the mean \pm S.E.M.

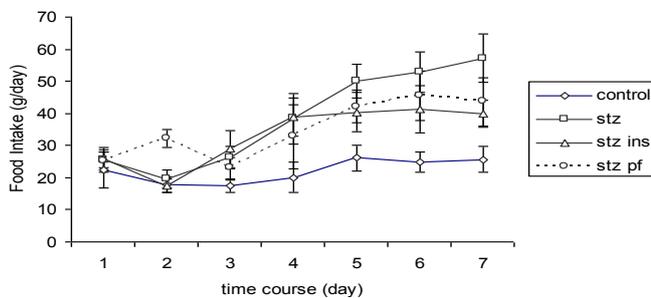
The behavioral effects of drug and vehicle treatments were evaluated statistically using the non-parametric Kruskal-Wallis analysis of

variance by runk, followed by the Mann-Whitney *U*-test with Bonferroni correction. Statistical significance was set at the $P < 0.05$ level.

Results

Effects of Insulin on Food Intake and Body Weight in STZ- Induced Diabetic Rats

We analyzed the changes in food intake and body weight over time. Figure 1 shows the changes in the daily food intake of STZ rats, which was greater than the control group ($p < 0.05$). There was a decrease in the STZ+INS group's food intake when compared with that of the STZ group ($p < 0.05$).



The data represent the mean \pm SD (n = 7 animals /group)
 * $P < 0.05$, compared to the control group.
 † $P < 0.05$ compared to STZ-induced diabetic groups

Figure 1. Changes of daily food intake in control, STZ pair-fed, Streptozotocin(STZ)-induced diabetic groups, Streptozotocin(STZ)-induced diabetic rats treated with insulin.

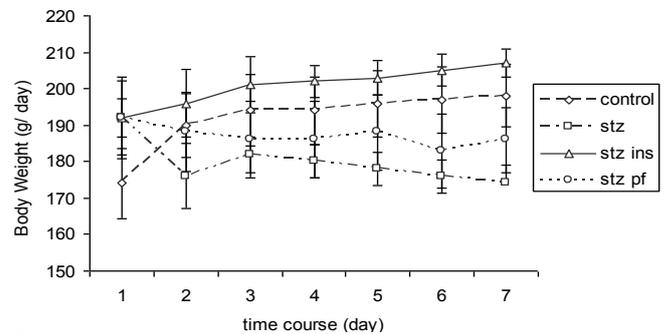
Effects of Insulin on Body Weight in STZ- Induced Diabetic Rats

There was also a decrease in the STZ group's body weight compared to control group ($p < 0.05$, Figure 2). The STZ+INS group had greater body weights compared with STZ and STZ PF groups ($p < 0.05$, Figure 2). In this study, insulin treatment had a significant effect on food intake and body weight at a dose range of 5-7 U over 7 days.

Effects of Insulin on Plasma Glucose in STZ- Induced Diabetic Rats

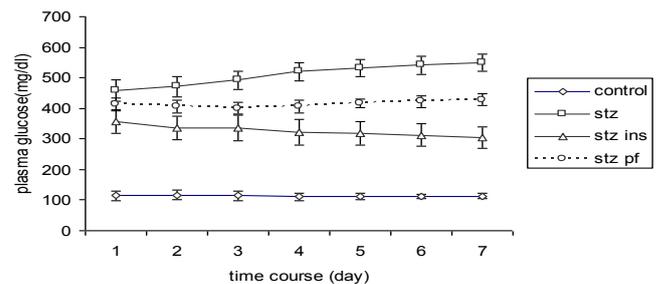
The changes in plasma glucose were analyzed over time; Figure 3 shows the changes in STZ rats. The STZ group had higher plasma glucose levels than the control group ($p < 0.05$), and treatment with insulin decreased plasma glucose compared with levels in the STZ and

STZ PF groups ($p < 0.05$ for each group, Figure 3).



The data represent the mean \pm SD (n = 7 animals /group)
 * $P < 0.05$, compared to the control group.
 † $P < 0.05$ compared to STZ-induced diabetic groups

Figure 2. Changes of body weight in the control, STZ pair-fed, Streptozotocin(STZ)-induced diabetic and Streptozotocin(STZ)-induced diabetic groups treated with insulin.



The data represent the mean \pm SD (n = 7 animals /group)
 * $P < 0.05$, compared to the control group.
 † $P < 0.05$ compared to STZ-induced diabetic groups

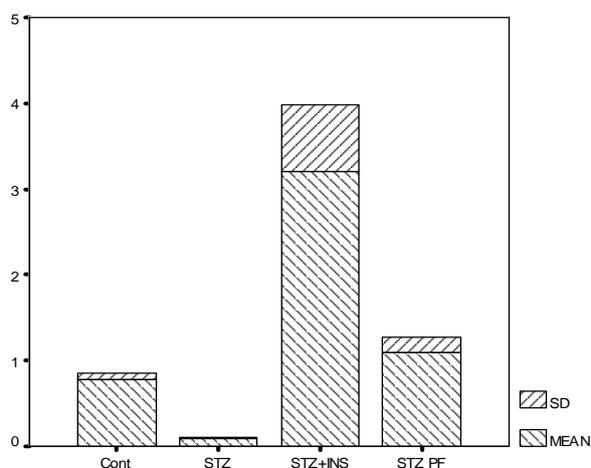
Figure 3. Changes of plasma glucose (mg/dl) in the control, STZ pair-fed, Streptozotocin(STZ)-induced diabetic and Streptozotocin(STZ)-induced diabetic groups treated with insulin.

Effects of Insulin Treatment on Plasma Insulin Levels

There was a significant difference in plasma insulin levels between the STZ and STZ+INS groups ($p < 0.05$, Figure 4).

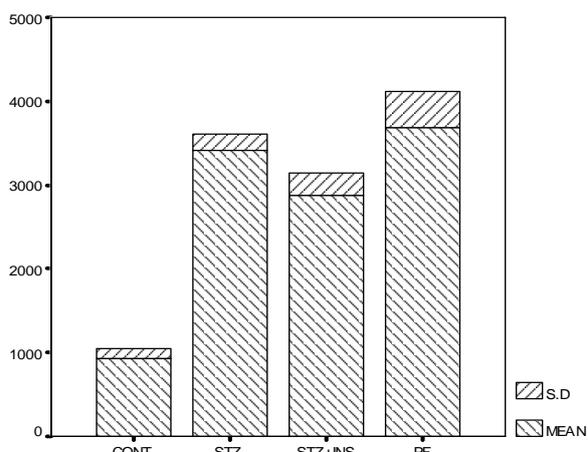
Effects of Insulin on Plasma Ghrelin Concentrations in STZ- Induced Diabetic Rats

Plasma ghrelin levels increased in the STZ group compared to the control and STZ+INS groups. Insulin treatment decreased plasma ghrelin levels compared with levels in the STZ group. ($p < 0.05$, Figure 5).



The data represent the mean \pm SD (n= 7 animals /group)
 * P<0.05, compared to the control group.
 † P<0.05 compared to STZ-induced diabetic groups and STZ pair-fed group

Figure 4. Changes of plasma insulin concentrations 7 days after induction of diabetes in the control, STZ pair-fed, Streptozotocin(STZ)-induced diabetic and Streptozotocin(STZ)-induced diabetic groups treated with insulin.



The data represent the mean \pm SD (n= 7 animals /group)
 * P<0.05, compared to the control group.
 † P<0.05 compared to STZ group

Figure 5. Changes of plasma ghrelin concentrations 7 days after induction of diabetes in the control group, STZ pair-fed group, Streptozotocin(STZ)-induced diabetic groups and Streptozotocin(STZ)-induced diabetic group treated with insulin.

Discussion

The hyperphagia at streptozotocin (STZ)-induced diabetic rats was accompanied by hyperglycemia and hypoinsulinemia.^{17,18} Plasma insulin levels are reduced in STZ induced

diabetic rats, and insulin deficiency is the main cause of hyperphagia in STZ induced diabetic rats because insulin plays a suppressive role in the central nervous system at food intake. In addition, previous studies have demonstrated that decreased plasma insulin levels and insulin secretion are related to hyperphagia in STZ-induced diabetic rats.^{17,19} Treatment of STZ-induced diabetic rats with insulin reversed these changes according to our results. A major new finding of this study is that plasma ghrelin levels were elevated in STZ –treated diabetic rats. Since ghrelin strongly promotes increased food intake in rodents and humans, these findings suggested that the high plasma ghrelin levels contribute to diabetic hyperphagia in rats. Negative energy balance in diabetic rats might induce a compensatory signal to upregulate ghrelin mRNA expression and to increase ghrelin secretion. Previous studies demonstrated that insulin suppresses endogenous ghrelin release in humans²⁰ and rats.²¹ Postprandial ghrelin remains increased in Type 1 diabetes when the patients do not receive insulin therapy.²² But Ferzli et al.²³ reported that plasma levels of ghrelin are decreased in patients with type 2 diabetes.

Recent research has reported that plasma ghrelin concentrations in untreated diabetic rats are significantly higher than levels in control rats^{13,17}, that elevated plasma ghrelin levels can contribute to diabetic hyperphagia by increasing hypothalamic NPY(neuropeptide Y), and that plasma ghrelin levels can be normalized using insulin treatment.¹³ The present study supported the previous researches indicating that plasma ghrelin concentration is significantly higher in STZ-induced diabetic rats than in control rats^{24,25}; however, the mechanism of elevation of ghrelin levels in STZ induced diabetes is largely unknown.

Therefore, the goal of our study was to clarify the regulation of plasma ghrelin by insulin in STZ induced diabetic rats. This study demonstrated that insulin treated rats improves blood glucose and food intake values, supporting a previous research.²⁶ There was difference in blood glucose levels between STZ-INS and STZ groups; and thus blood glucose levels were higher in the STZ-INS group than in the control group. In this study, the STZ group had higher plasma ghrelin levels compared to control group. Food intake as well as blood ghrelin were

reduced at insulin treated rats. However, we cannot rule out the involvement of glucose or insulin in the regulation of ghrelin in STZ rats. Further studies are needed to examine active ghrelin as well as full ghrelin.

Conclusion

This study demonstrated that insulin improved weight loss in STZ-induced diabetic rats, even in a pair-fed condition. These results may be due to changes in hyperglycemia and/or insulin action on body weight caused by the insulin treatment. In conclusion, we demonstrated that insulin regulates the suppression of food consumption in STZ induced diabetic rats and can reverse the elevated plasma ghrelin levels.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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